Complete mitochondrial genome of a woolly mammoth (*Mammuthus primigenius*) from Maly Lyakhovsky Island (New Siberian Islands, Russia) and its phylogenetic assessment

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Abstract

We present a complete sequence and an annotation of the mitochondrial genome of the woolly mammoth (*Mammuthus primigenius*) found in 2012 on Maly Lyakhovsky Island (North-Eastern Siberia, Russia). The genome was 16,851 bp long and contained 13 protein-coding, 22 tRNA, and 2 rRNA genes. It was AT reach (61.3%) with A = 32.9%, T = 28.4%, C = 25.3%, and G = 13.4%.

Keywords

Ancient DNA, Elephantidae, North-Eastern Siberia, mitogenome, Maly Lyakhovsky Island

Main text

A partial carcass of a woolly mammoth female adult (*Mammuthus primigenius*) was found on the Maly Lyakhovsky Island (74°13'N, 141°03'E) of the New Siberian Islands archipelago in 2012. The remains contained relatively well-preserved soft tissues in some parts of the body (Grigoriev et al. 2017). The anatomy, morphology, hematology, histology and composition of intestinal microbal communities of this specimen were described in Grigoriev et al. (2017). To examine the level of DNA integrity in the specimen, and to study its phylogenetic relationships with other woolly mammoths, the tissue samples were collected for DNA extraction and sequencing of complete mitochondrial genome.

Samples of trunk muscle tissue were taken with sterile scalpel and scissors. Bone fragments were taken from a brachial bone and a rib. DNA isolation from each sample was performed using the PrepFiler BTA Forensic DNA Extraction Kit (Applied Biosystems / Thermo Fisher Scientific), DNA IQ System (Promega Corporation) and phenol-chloroform purification with modifications. An appropriate negative control was used at every step. The detailed DNA extraction protocol is provided in supplementary material.

PCRs were carried out in GeneAmp PCR System 9700 (Applied Biosystems, USA) using a set of primers covering whole mitochondrial genome and specifically designed for this study using Primer3 program (Untergasser et al. 2012). The amplification conditions and primer sequences are provided in supplementary material. Sequencing was performed on an ABI 3130XL automatic sequencer (Applied

Biosystems) using the Big-Dye®Terminator 3.1 kit (Applied Biosystems) and the same primers that were used for PCR amplification.

All available complete and partial mitochondrial genomes of other mammoths were downloaded from GenBank, aligned using Clustal Omega (Sievers et al. 2011) and trimmed manually for repetitive regions and gaps. The phylogenetic trees were generated using the maximum likelihood (ML) method and IQ-TREE 1.6.3 (Nguyen et al. 2015) with 1,000 ultrafast bootstrap replicates.

We were able to amplify the fragments up to 1,500 bp long (Figure S2 in supplementary material) confirming a relatively good DNA preservation considering the age of the specimen (32480-32930 years; Grigoriev et al. 2017). To the best of our knowledge, the only study, where similar level of amplification efficiency was achieved for aDNA, was performed on genomic DNA extracted also from the muscle tissue of a mammoth (Rogaev et al. 2006).

The mitogenome of our *M. primigenius* specimen was 16,851 bp long (NCBI GenBank accession number MF770243) and based on annotation included 13 protein coding, 22 tRNA and 2 rRNA genes in a typical vertebrate gene order. Short tandem repeats $(ACGCAT)_{\geq 40}$ were found between conserved sequence block 1 (CSB1) and CSB2 of the control region. It is difficult to estimate certain number of repeats due to possible polymerase stuttering or real heteroplasmy (Rogaev et al. 2006).

Woolly mammoths belonging to the two major phylogenetic lineages are known to occur in North-Eastern Siberia (Chang et al. 2017). The studied specimen was clustered together with the members of "Clade 1" (as defined in Chang et al. 2017; Figure 1).

Disclosure statement

The authors report no conflicts of interest.

References

Chang D, Knapp M, Enk J, Lippold S, Kircher M, Lister A, MacPhee R, Widga C, Czechowski P, Sommer R, et al. 2017. The evolutionary and phylogeographic history of woolly mammoths: A comprehensive mitogenomic analysis. Sci Rep. 7:1–10. https://doi.org/10.1038/srep44585

Grigoriev SE, Fisher DC, Obadă T, Shirley EA, Rountrey AN, Savvinov GN, Garmaeva DK, Novgorodov GP, Cheprasov MY, Vasilev SE. 2017. A woolly mammoth (*Mammuthus primigenius*) carcass from Maly Lyakhovsky Island (New Siberian Islands, Russian Federation). Quat Int. 445:89–103. https://doi.org/10.1016/j.quaint.2017.01.007

Kalyaanamoorthy S, Minh BQ, Wong TFK, von Haeseler A, Jermiin LS. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat Methods. 14:587-589. https://doi.org/10.1038/nmeth.4285

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2017. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol Biol Evol. 32:268-274. https://doi.org/10.1093/molbev/msu300

Rogaev EI, Moliaka YK, Malyarchuk BA, Kondrashov FA, Derenko MV, Chumakov I, Grigorenko AP. 2006. Complete mitochondrial genome and phylogeny of Pleistocene mammoth *Mammuthus primigenius*. PLoS Biol. 4:e73. https://doi.org/10.1371/journal.pbio.0040073

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, et al. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 7(1):539. http://www.doi.org/10.1038/msb.2011.75

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. 2012. Primer3 — new capabilities and interfaces. Nucleic Acids Res. 40(15):e115 https://doi.org/10.1093/nar/gks596